INTERACTION BETWEEN
HEART RATE AND CALCIUM CONCENTRATION IN THE
CONTROL OF CONTRACTILE STRENGTH OF
THE FROG HEART

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SUMMARY

1. The changes in twitch tension during the ascending and descending staircase of the frog heart have been examined under various experimental conditions including the hypodynamic and prehypodynamic state.

2. The descending staircase resembles the tension change after reduction of external calcium concentration in showing two consecutive phases, an initial rapid and later slow phase of tension decline.

3. The time course of the ascending staircase depends on the condition of the heart; it is slow after, and rapid before, development of the hypodynamic state. It is also rapid when elicited after conditioning periods of increased heart rate.

4. The tension transients in response to brief concentration steps of external calcium concentration were examined at various levels of heart rate. The results indicate that at high heart rates the sensitivity of heart cells to external calcium is increased.

5. The results are interpreted along the lines of a previous hypothesis relating tension development to the cooperative action of two intracellular calcium compounds. The additional assumption is made that an intermediary exists which facilitates calcium movements in heart cells to an extent depending on the level of heart rate.

INTRODUCTION

Results described in the preceding paper (Chapman & Niedergerke, 1970) suggest that contraction of the frog heart is brought about by the action of two different intracellular calcium compounds, Ca_1 and Ca_2,

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which both vary with the external calcium concentration but respond at characteristically different rates to a change in this concentration. Compound Ca_1 appears to initiate the contractile process, whereas Ca_2 modifies its strength; the response to a change in external calcium is much more rapid for Ca_1 than for Ca_2, as is indicated by the two phases of tension change obtained under these conditions.

In the present paper this dual control of contractile activity has been further examined in experiments in which both the heart rate and level of external calcium were altered. It was found that even in the simplest case, when only the heart rate was varied, both the 'ascending' and 'descending' staircase responses obtained were often composite, consisting of a rapid followed by a slow phase. The time course of the slow phase of tension change closely resembled that observed on altering external calcium, suggesting that changes in the concentration of the compound Ca_2 also occur in this situation. The presence of the fast phase was however suggestive of a change in the level of Ca_1, although the time course of this phase differed from that obtained after variation of external calcium.

To analyse this complex situation further we have examined the tension transients evoked by brief changes of external calcium concentration in the presence of either low or high levels of heart rate. The outcome of these experiments may be summarized by saying that the responsiveness of heart cells to the effects of external calcium is enhanced when the heart rate is high.

In a tentative hypothesis made to interpret these findings it is assumed that high levels of heart rate facilitate the inward movement of calcium ions and thereby increase the concentrations of both compounds Ca_1 and Ca_2 inside the cells. A model along these lines is described.

METHODS

The methods have been described in the preceding paper (Chapman & Niedergerke, 1970).

RESULTS

The time course of the ascending and descending staircase

When the rate at which an isolated heart is stimulated is changed, the twitch tension responds in a characteristic way, becoming larger with increasing rate ('ascending staircase') or smaller ('descending staircase') when the rate is diminished. These staircase responses have much in common with the changes brought about by variations of the external calcium concentration described in the preceding paper (Chapman & Niedergerke, 1970), and the results of the first sections of the present paper

illustrate the similarity of the two types of responses. A later section is concerned with the analysis of certain differences which have also been observed.

The dependence of the ascending staircase on the degree of the hypodynamic condition. Fig. 1b shows two staircases, curves A and B (represented as continuous lines through the peaks of tension traces of twitches), which
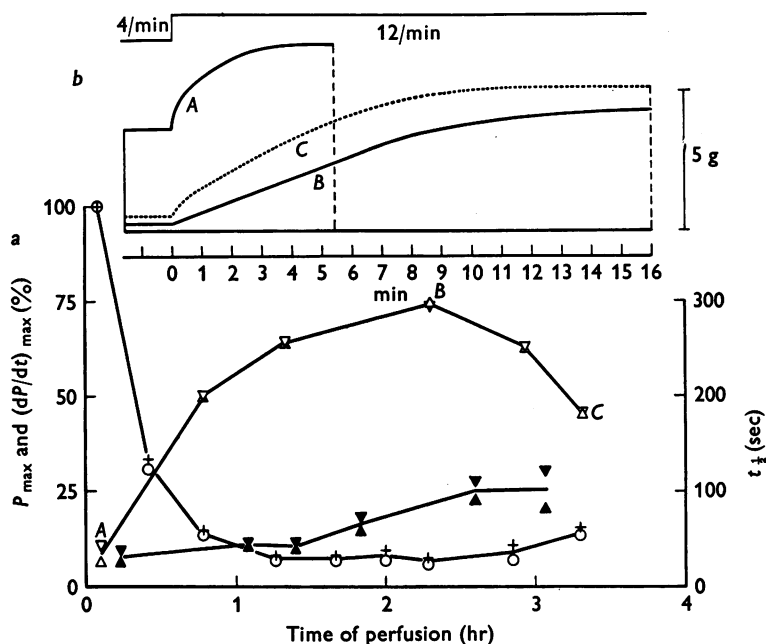

Fig. 1. The staircase of contraction during development of the hypodynamic condition. *a.* Hypodynamic changes in a ventricle after commencement of perfusion with Ringer fluid (1 mM-Ca-Ringer throughout) (1) of twitch heights at constant low heart rate (4 min^{-1}), ○ peak tension, P_{max}, + maximum rate of rise of tension, $(dP/dt)_{max}$, (both as % of the first pair of values determined; L.H.S. ordinate); (2) of the half-times of ascending staircases induced by enhancement of the heart rate from 4 to 12 min^{-1}, △ from traces of P_{max}, ▽, from traces of $(dP/dt)_{max}$; (3) of the half-times of descending staircases (heart rate reduced from 12 to 4 min^{-1}), ▲ from traces of P_{max}, ▼ from traces of $(dP/dt)_{max}$ ((2) and (3) R.H.S. ordinate). *b.* Profiles of three ascending staircases (lines through levels of P_{max} of tension traces) of experiment illustrated in *a*: A at the commencement of experiment, B more than 2 hr afterwards, C at the end of experiment.

were both obtained after enhancement of the stimulus rate from 4 to 12 min^{-1}, in the first case before, and in the other after, complete development of the hypodynamic condition. As is well known (cf. Clark, 1913) the ascending staircase of the fresh heart started from a high level of tension and was short (curve A) whereas the staircase in the hypodynamic con-

dition starting from a low level of tension was much longer (curve B). Fig. 1a, in a more complete illustration of this experiment, shows that the hypodynamic weakening of heart beats was accompanied by a progressive increase in the half-time of the ascending staircase, in the same way as was found to be associated with the increase in half-time of high-calcium induced tension rise (cf. Fig. 2a, Chapman & Niedergerke, 1970). Two
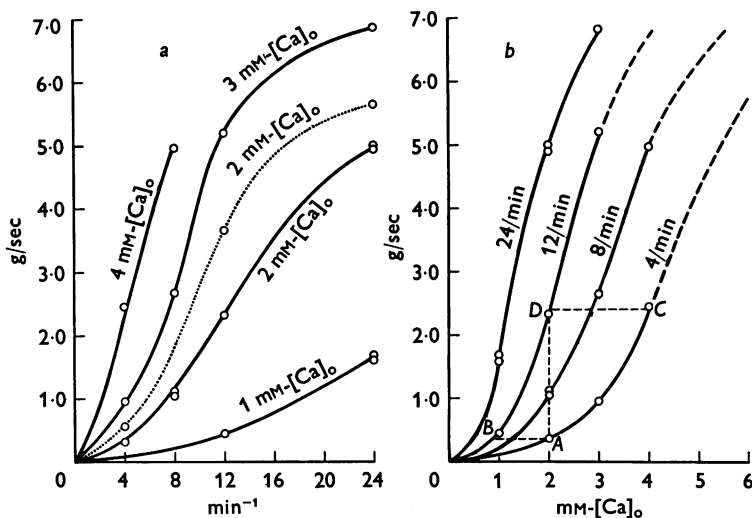

Fig. 2. Twitches of ventricle equilibrated to various values of external calcium and heart rate. a. Levels of $(dP/dt)_{max}$ plotted against stimulus frequency. Points connected by continuous lines obtained after, those by dotted line before, completion of development of the hypodynamic condition. b. Re-plot of the values of $(dP/dt)_{max}$ in a against $[Ca]_o$. (Results obtained before completion of development of the hypodynamic condition have been omitted; for the significance of points A to D and the lines connecting them, see text.)

further points illustrated in Fig. 1a should be mentioned: (a) changes in time course of the descending staircase were much smaller than those of the ascending staircase just described, although a gradual increase of the half-time of this tension decline was usually observed, as in the present case. (b) Certain of the effects of the hypodynamic condition, e.g. both the decline in twitch tension and the slowing of the ascending staircase, frequently diminished towards the end of long experiments (as is shown by the comparison of curves C and B in Fig. 1b). This phenomenon, which is probably associated with a deterioration of the preparation, has not been further examined, and experiments were usually terminated at the first sign of its appearance.

Fig. 2a shows values of $(dP/dt)_{max}$ of twitches recorded in an experiment in which both the steady heart rate and external calcium concen-

tration were altered. Values were determined with the preparation in the hypodynamic state (those in 2 mM-Ca-Ringer were obtained, both at an early stage of, and after, development of this condition). As is seen, the slope of the S-shaped frequency–response curve increased with increasing levels of $[Ca]_0$ but declined as a result of the development of the hypodynamic condition (cf. also Hajdu & Leonard, 1961). To aid further comparison of the effects of variation in heart rate and external calcium the points of Fig. 2a have been replotted in Fig. 2b against levels of $[Ca]_0$. It is convenient to use this illustration to describe some features of the procedure employed in the subsequent experiments. In these, tension changes were examined over a range of tension usually not much above the level of the line *DC* in Fig. 2b so as not to exceed the region of the steepest rise in each of the 'dose–action' curves, but also not below the level *BA* to avoid complex effects which occur when the heart rate or calcium concentration, or both, are low, as described recently by Brown & Orkand (1968). The experiments were also limited to the period after the initial 1–2 hr of perfusion was over and heart ventricles had passed into the hypodynamic condition.

Comparison of the time course of tension decline due to reduction of either $[Ca]_0$ *or the heart rate.* In terms of Fig. 2b the procedure in these experiments (cf. Fig. 3, curves *A* and *B*) was to increase twitch heights from a low steady level ψ_1 (indicated by point *A*) to a constant upper level ψ_u (points *C* or *D*) by enhancement either of the heart rate (from 4 to 12 min^{-1}) at a constant level of $[Ca]_0$, or of $[Ca]_0$ (which was increased from 2 to 4 mM in Fig. 2 or from 3 to 6 mM in Fig. 3), at a constant heart rate. Subsequently, the heart rate or $[Ca]_0$ was reduced to the original low level, and the results of tension decline for both these cases were plotted in Fig. 3b (curves *B* and *A*) and analysed in the same way as was described in connexion with Fig. 7 of the previous paper (Chapman & Niedergerke, 1970). Comparison of the two curves so obtained shows that tension decline of the descending staircase proceeded, like the decline in low calcium fluid, in two consecutive, approximately exponential, phases described by eqn. (1)

$$(\psi - \psi_1) = \Delta\psi \exp(-t/\tau_1) + (\psi_1 - \psi_1)\exp(-t/\tau_2), \tag{1}$$

where the significance of the symbols chosen is the same as that of eqn. (1) (Chapman & Niedergerke, 1970), i.e. ψ_1 represents the level of tension at the intercept with the ordinate of the slow phase of tension decline, and the initial magnitudes of fast and slow phases are $\Delta\psi (= \psi_u - \psi_1)$ and $(\psi_1 - \psi_1)$, respectively. With regard to the values of the time constants of the two curves under comparison, those of τ_2, of the slow phase of tension decline, were closely similar, i.e. 157 sec (S.E. ± 8.7, $n = 13$) and 147 sec

(S.E. ± 8.9, $n = 13$) after reduction of heart rate and $[Ca]_o$ respectively. However, a significant difference was apparent for τ_1 of the fast phase, which was larger after reduction of heart rate than of $[Ca]_o$, 17.8 sec (S.E. ± 2.9, $n = 12$) as against 10.5 sec (S.E. ± 0.6, $n = 12$) (both values being obtained at about $22°$ C). The difference was particularly marked in experiments made at a reduced temperature, of e.g. $12°$ C, and this is related to the fact that values of τ_1 obtained from the descending staircase, but not the low calcium induced tension decline, increased in response to
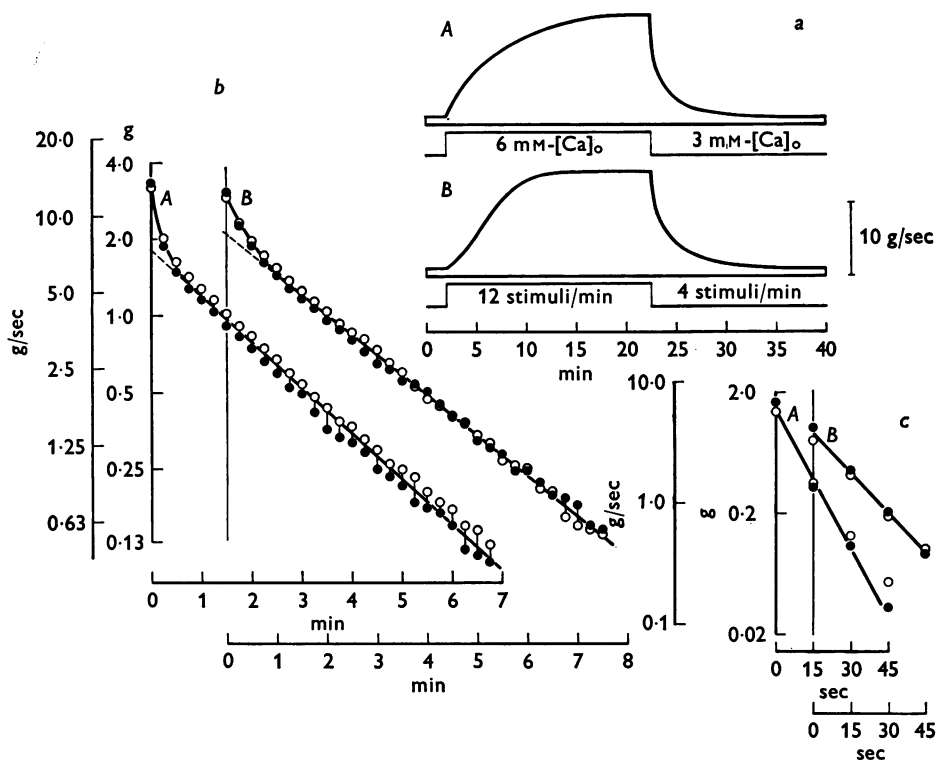

Fig. 3. Time course of tension decline after reduction either of $[Ca]_o$ or of heart rate. *a*. Build-up and decline of twitches over identical range of values of $(dP/dt)_{max}$ (A) due to variation of $[Ca]_o$ from 3 to 6 and back to 3 mM (at constant heart rate of 4 min^{-1}) and (B) due to variation of heart rate from 4 to 12 and back to 4 min^{-1} (at constant 3 mM-$[Ca]_o$). (Traces represent smooth lines drawn through peaks of differentiated twitch records.) *b*. Semi-logarithmic plot of the decline of values of P_{max}, $\bigcirc$, and of $(dP/dt)_{max}$, $\bullet$, against the time after reduction of $[Ca]_o$ (A), or of heart rate (B). (Procedure as in Fig. 7b, Chapman & Niedergerke, 1970). *c*. Semi-logarithmic plot of phase of rapid decline of P_{max}, $\bigcirc$, and of $(dP/dt)_{max}$, $\bullet$, after reduction of $[Ca]_o$ (A) or of heart rate (B). Points obtained from the difference between measured values $(\psi - \psi_1)$ and those obtained by extrapolation to zero time of values of either P_{max} or $(dP/dt)_{max}$.

reduction of the temperature (about threefold for a temperature step of $-10°$ C).

The results summarized by eqn. (1) can be interpreted along the lines of the hypothesis of the preceding paper by saying that enhancement (or reduction) of the heart rate causes the accumulation (or decline) of intra-cellular calcium and hence a change in level of the slow compound Ca_2 in much the same way as does an alteration in external calcium concentration. This is suggested both by the similar time course of the slow tension changes in the two curves under comparison and by the recent demon-stration that conditions of varying heart rate are associated with net cal-cium movements, which have a similar time course to those of the slow tension changes just described (Niedergerke, Page & Talbot, 1969 *a, b*, and unpublished). In a similar way, the presence of a phase of rapid tension decline suggests that the staircase is also accompanied by a concentration change of compound Ca_1, although the mechanism of this change probably differs in certain important details from that of the change of Ca_1 which occurs when the external calcium concentration is changed. This will be discussed in greater detail in connexion with Figs. 10 and 11.

Further analysis of descending and ascending staircases. This hypothesis has been tested in three series of experiments illustrated in Figs. 4, 5 and 6 with procedures similar to those of the corresponding experiments in the preceding paper (cf. Figs. 7, 12 and 14, Chapman & Niedergerke, 1970) in which the level of $[Ca]_0$ rather than the heart rate was altered. In the first of these experiments which is analogous to Fig. 7 (Chapman & Niedergerke, 1970) and illustrated in Fig. 4, a series of ascending staircases were induced from a constant low tension level, ψ_1, on each occasion by increasing the heart rate from 4 to 12 min^{-1}. The resulting build-up of the heart beats was interrupted at various tension levels, ψ_u, by a descending staircase initiated by reduction of the heart rate to the original value of 4 min^{-1}. The family of curves of descending staircases from one such experiment (cf. Fig. 4a) shows that the rise of tension level ψ_u was accompanied by a rise of level ψ_1, as indicated by the upward shift of both the points of origin of the curves at the ordinate and those at the intercept with the ordinate of the extra-polated slow phases of tension decline. In Fig. 4c, in which the results of several experiments are combined in a plot of values of $(\psi_u - \psi_1)$ versus $(\psi_1 - \psi_1)$, it is seen that the two tension levels run approximately in pro-portion to each other, except in one experiment in which the level ψ_1 at the commencement of the ascending staircase was high. (It may be noted that in this case the rise of ψ_u had been more rapid, relative to that of ψ_1, for short as compared to long ascending staircases; see points connected by interrupted line and compare with the results of a similar experiment in Fig. 7d, Chapman & Niedergerke, 1970). The other, related, finding is that

the magnitude of $\Delta\psi$ (the rapid tension decline) also increased with in-creasing levels of ψ_u, as is illustrated by the progressive upward shift under these conditions of the curves fitted to the phases of rapid tension change in Fig. 4*b* (compare with Fig. 7*c*, Chapman & Niedergerke, 1970). The similarity of these results to those obtained in response to variation of [Ca]$_0$ (cf. Fig. 7, Chapman & Niedergerke, 1970) clearly supports the initial hypothesis in suggesting that the increase in level of [Ca$_2$] is the
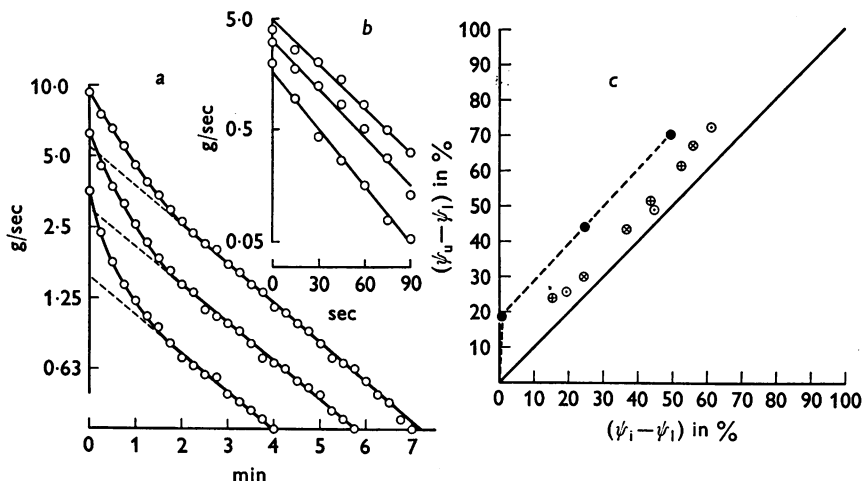

Fig. 4. Time course of descending staircases following ascending stair-cases of varying heights. Change of heart rate in each case from 4 to 12 min^{-1} and back to 4 min^{-1}. *a*. Semilogarithmic plot of tension decline of descending staircases (procedure as in Fig. 3*b* except that only values of $(dP/dt)_{max}$ are plotted). Build-up of tension had either been complete, or 67 % or 39 % complete (corresponding to sequence of curves from top to bottom). *b*. Semilogarithmic plot of initial phase of tension decline of curves in *a* (procedure as in Fig. 3*c*). *c*. Combined results of four experi-ments of the type illustrated in *a*. Values of $(\psi_u-\psi_1)$ plotted against $(\psi_1-\psi_1)$ (cf. eqn. 1). Ordinate and abscissa expressed in % of average values of $(\psi_u-\psi_1)$ and $(\psi_1-\psi_1)$ obtained from two descending staircases following 'complete' ascending staircases and induced before and after each 'incom-plete' staircase. mM-[Ca]$_0$: $\oplus$ 1, $\otimes$ 1·5, $\odot$ and $\bullet$ 2. In experiment $\bullet$ tension level ψ_1 was relatively high, i.e. about 20 % of ψ_u of 'complete' staircase (cf. also discussion in conjunction with Figs. 7*d* and 13, Chapman & Niedergerke, 1970).

cause of slow tension rise (in the present case of the ascending staircase) and at the same time has the effect of amplifying the phase of rapid tension change (in this instance of the descending staircase).

The next series of two experiments, illustrated in Figs. 5 and 6, were both concerned with the dependence of the time course of the ascending staircase on the level (ψ_1) of tension developed before the heart rate was

increased, i.e. before commencement of the staircase. In the experiment of Fig. 5, this level was set by prior adjustment of the heart rate to a point within the range 4–12 min^{-1}; when a stable response was obtained the rate was increased, in every case to an upper level of 24 min^{-1} so that the final height of the staircase was constant throughout. From the series of ascending staircases obtained in this experiment (see Fig. 5a) and the
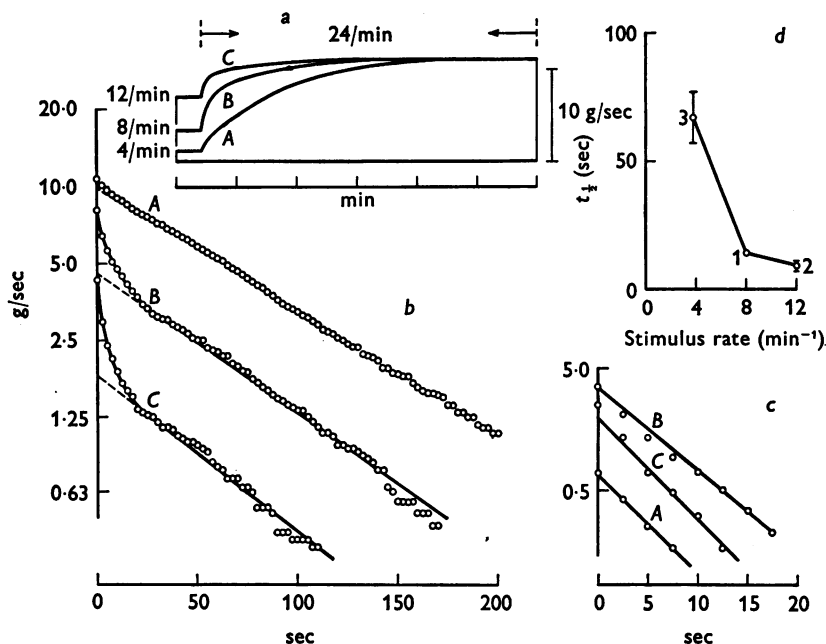

Fig. 5. Ascending staircases induced by increasing the heart rate from a series of low values (4 min^{-1}, 8 min^{-1} and 12 min^{-1}) to a constant upper level, of 24 min^{-1}. [Ca]$_0$ kept constant at 2 mM throughout. a. Profiles of three staircases (as in Fig. 3a). b. Plot of the log of values ($\psi_{t=\infty} - \psi$) (from records of $(dP/dt)_{max}$) against time after commencement of each staircase. Letters refer to the same curves as in a. c. Semilogarithmic plot of the initial phases of the ascending staircases in b. d. Dependence of the half-time of the ascending staircase on the stimulus rate before onset of the staircase. Numbers refer to number of ventricles, each of which provided several values, with a range shown by the length of the vertical bars.

half-time of build-up of these staircases (see Fig. 5d) it is clear that tension build-up was rapid when the initial level of tension was high. The curves of tension rise in Fig. 5b obtained from the plot of the difference ($\psi_{t=\infty} - \psi$) against the time after onset of each staircase shows that this effect was associated with, and largely due to, the growth of an initial phase of rapid tension rise (cf. Fig. 5d). In the other type of experiment (cf. Fig. 6) ascending staircases, each induced by enhancement of the heart rate from

4 to 12 min^{-1}, were made to start from various levels to which tension had declined during a preceding descending staircase (in response to the reduction of heart rate from 12 to 4 min^{-1}). The results have been plotted in Fig. 6a, b and c in a similar way to those of Fig. 5a, b and c, and again
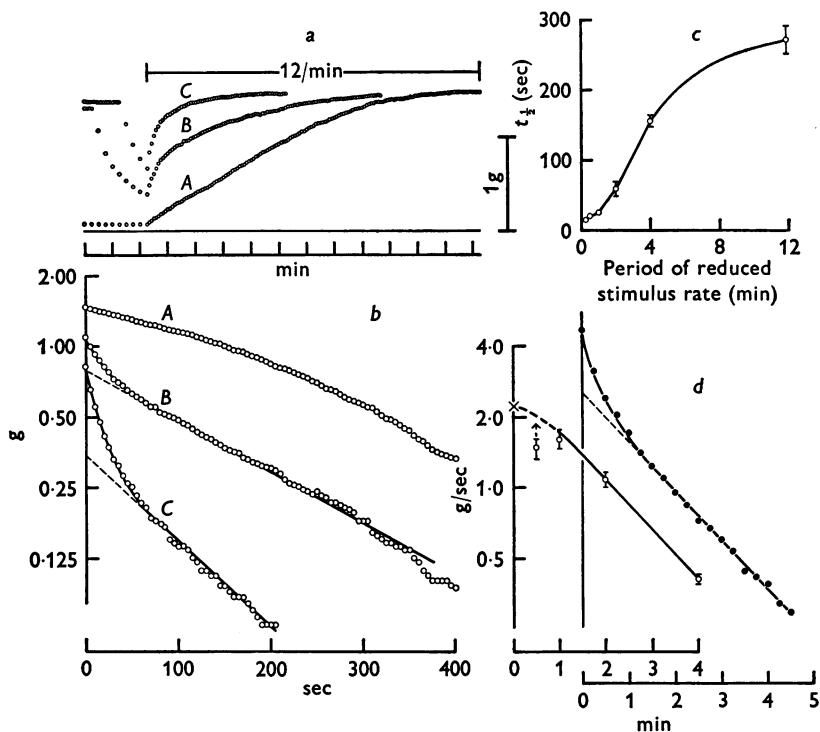

Fig. 6. Ascending staircases induced by enhancement of the stimulus rate from 4 to 12 min^{-1} after descending staircases (due to reduction of this rate from 12 to 4 min^{-1}) of varying durations; [Ca]$_o$ constant at 2 mM. a. Three tracings each of an ascending staircase (values of P_{max}) either (A) 20 min, (B) 2 min or (C) 1 min after onset of descending staircase. b. Semilogarithmic plot of the three ascending staircases illustrated in a (plotted as in Fig. 5b). c. Half-times of the ascending staircases of this experiment plotted against durations of the preceding descending staircases. d. L.H.S. curve: semilogarithmic plot of initial phases of rapid tension build-up against duration of the preceding descending staircases. (Arrow at 30 sec is a correction indicating the extent to which the phase of rapid tension rise may be underestimated because of its superposition upon the preceding phase of rapid decline. The length of the arrow, from the point of the average value of rapid build-up, is equal to the residual magnitude of rapidly declining tension at the moment of commencement of tension build-up; × initial magnitude of rapid tension decline). R.H.S. curve: semilogarithmic plot of tension decline of descending staircase from the same experiment. Note the identical slope of the later portion of the two curves.

show that the staircases starting from a high level of tension were short and included a large phase of rapid tension rise. For further analysis, the initial magnitude of this phase, $\Delta\psi_b$, was determined from each build-up curve by means of the usual procedure (cf. Fig. 3c and Figs. 5c) and the values of $\Delta\psi_b$ so obtained have been plotted in Fig. 6d against the time during which the heart rate was low (L.H.S. curve). The R.H.S. curve of Fig. 6d is the logarithmic plot of the descending staircase recorded in the same experiment after reduction of the heart rate from 12 to 4 min^{-1}. As is seen, the straight lines fitted to the later portions of both these curves have identical slopes, that of the phase of slow tension decline.

Comparison of Figs. 5a, b and d and 6a, b and c with Figs. 12a, b and c and 14a, b and c of the preceding paper (Chapman & Niedergerke, 1970) reveals a further point of similarity between the results obtained after enhancement of either calcium concentration or heart rate. In particular, the fact that in the experiment of Fig. 6d the magnitude of the rapid component of tension build up declined with time at a rate similar to that of the slow phase of the descending staircase suggests, by analogy with the interpretation of the result of Fig. 14c (Chapman & Niedergerke, 1970), that in the present case, as before, high levels of [Ca$_2$] serve to amplify the rapid tension changes which, in turn, are attributed to an effect of a change in the concentration of Ca$_1$. (This change in [Ca$_1$] may be presumed to have been constant in the experiment of Fig. 6 because of the use of a constant step in heart rate, from 4 to 12 min^{-1}.)

Some further aspects of the time course of the slow phase of the ascending staircase also require comment. Although in the experiment of Fig. 5b this phase could be satisfactorily approximated by a single exponential, more complex shapes were found in other experiments, especially in those in which the level at the start of the staircase was low. The staircase was then either linear for much of its build-up (cf. curve A of Fig. 6a) or included a portion of very shallow rise or even a decline of tension (cf. Brown & Orkand, 1968). These features and others, e.g. that the rate of slow tension rise often increased with increasing levels of initial tension (cf. Fig. 6b), are incompletely understood and have not been further examined.

Further analysis of rapid tension changes

For the purposes of a more detailed analysis it was convenient to use a different, though related, procedure to that described in the preceding sections. The experiments were begun by application of stimuli at a constant rate (e.g. 12 min^{-1} in the experiment of Fig. 7) to obtain twitches of constant amplitude; this achieved, stimulation was interrupted for various intervals. The size of the first beat on renewing stimulation at the same rate as before then served as a measure of the level to which contractility had declined during preceding quiescence. A duplicate series of declining twitch heights obtained in this way has been plotted in Fig. 7a against the

length of the quiescent intervals, and the semilogarithmic plot of these results in Fig. 7b shows that tension decline again occurred in two consecutive phases and with a time course similar to that of the descending staircase which has been plotted for comparison in Fig. 7c. Thus, the half-times of the two phases, separated in the same way for each of the two
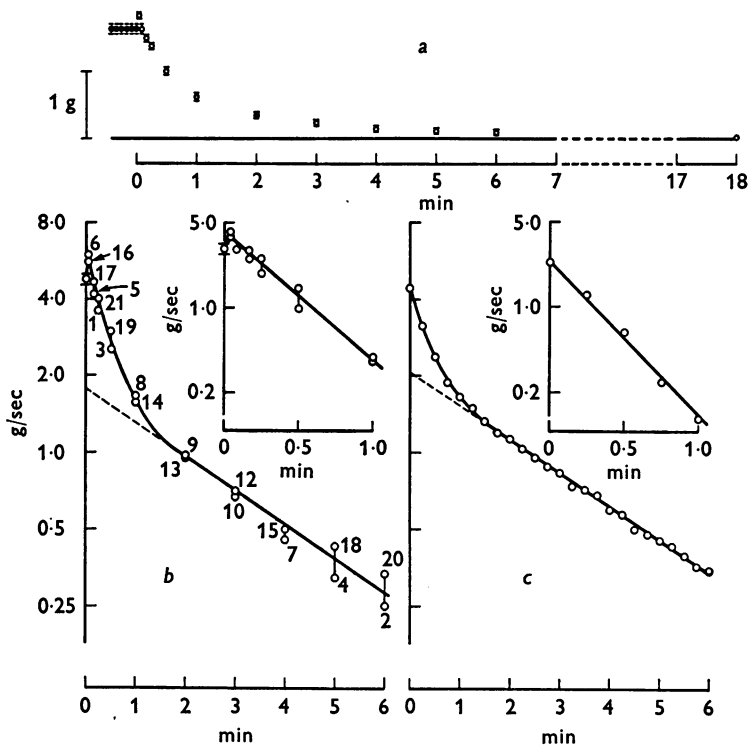

Fig. 7. Decline of contractility during periods of quiescence after continuous stimulation. a. Twitch heights (values of P_{max}) during continuous stimulation at the rate of 12 min^{-1} ● (average of twenty-three determinations) and after cessation of stimulation and application of single test shocks ○ (each point average of two determinations). Distance between horizontal bars: range of values of P_{max}. 2 mM-[Ca]$_o$ throughout. b. Semilogarithmic plot of the declining twitch heights (values of $(dP/dt)_{max}$) and (inset) of the initial phase of rapid decline (procedure as in Fig. 3c). Numbers indicate sequence of tests during experiment (each point is the result of a single twitch). c. Semilogarithmic plot of a descending staircase (the response to reduction of heart rate from 12 to 4 min^{-1}) recorded in the same experiment. Inset: initial phase of rapid tension decline.

curves in Figs. 7b and c, were in both cases 12 sec and 135 sec, respectively (although the exact agreement of the two sets of values in this experiment is probably fortuitous). It may be noted that the first twitch after continuous stimulation (e.g. in Fig. 7a) was stronger than the preceding

twitches, a result which is readily understood because the test pulse in this case was applied after an interval (of 2·5 sec) shorter than the beat interval (of 5 sec) during stimulation and, hence, at an earlier point in the 'super-normal' phase of contractility which follows each twitch.

The method just described was also applied in a further study of the fast phase of the ascending staircase. An experiment of this series is illustrated in Fig. 8. Here a short (ascending) staircase was induced by three
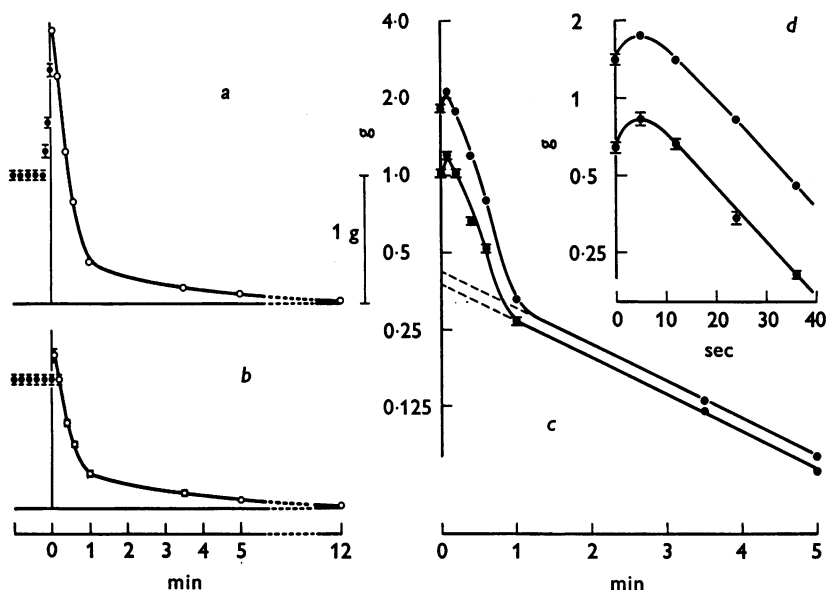

Fig. 8. Decline of contractility during quiescence either after regular stimulation at a constant rate or after build-up of a short staircase. *a* and *b*. Tracings of twitches (values of P_{max}) ● during preliminary period of continuous stimulation (rate 5 min^{-1}), including in *a*, but not in *b*, a short staircase (three twitches at the rate of 15 min^{-1}), and ○ after cessation of stimulation in response to single test shocks. 2 mM-$[Ca]_o$ throughout. *c*. Semilogarithmic plot of the height of 'test' twitches evoked after various periods of quiescence: lower curve, after continuous stimulation (results from *b*), upper curve, after additional short staircase (results from *a*). (*d*) Semilogarithmic plot of the two initial phases of rapid tension decline. Separation of horizontal bars in *c* and *d*: range of values obtained.

stimuli, at the rate of 15 min^{-1}, following continuous stimulation at the lower rate of 5 min^{-1} (cf. Fig. 8*a*). The time course of tension decline after subsequent periods of quiescence was compared with that determined in the same way in the absence of the short staircase (Fig. 8*b*). As is seen from the two sets of curves obtained after the usual separation of the two consecutive phases (Figs. 8*c*, *d*), the effect of the short staircase had been to enhance greatly the phase of rapid tension decline whereas the phase of

slow decline was increased to only a small extent (the former by more than 100 %, as indicated by the displacement of the maximum of the curves in Fig. 8d from a level of 0·8–1·8 g, the latter by about 12 %, as indicated by the upward shift, from 0·38 to 0·42 g, of the point at which the extrapolated slow phase intercepts the ordinate in Fig. 8c). This result, the rapid decay of a component of tension which had previously built up at a rapid rate, is reminiscent of a similar result obtained after transient enhancement of external calcium concentration (cf. Figs. 13 and 15, Chapman & Niedergerke, 1970) and is suggestive of the occurrence of the same process, i.e. a change in level of $[Ca_1]$.

With regard to the mechanism underlying this change in level of $[Ca_1]$, two hypotheses come to mind. According to the first, a fraction of the calcium which moves into and out of the cells during activity is left behind after each twitch in a certain region of the cell, e.g. at the outer cell surface after extrusion of the ion from the cells, or as a residual amount of Ca_1 inside the cells, and disperses in the external or intracellular medium with the same time course as that of rapid tension decline. Given a short enough beat interval, these residual quantities of calcium could accumulate and so give rise to the progressive build-up in level of $[Ca_1]$. In the other hypothesis the cumulative event does not primarily involve calcium, at any rate not at the external membrane surface (though, conceivably, within the cell membrane), but an agent which controls the movement of calcium ions into the cells. It might be imagined, for example, that an intermediary, I, which is formed during or after each action potential and subsequently rapidly declines, has the property of facilitating inward movement of calcium either through the cell surface or further inside the cell. Clearly, accumulation of this compound could also bring about a rise in level of $[Ca_1]$.

In experiments made to test these and other possibilities, the effects of transient changes of external calcium on the strength of twitches were examined in two conditions: (a) at a steady low heart rate and (b) after the build-up of a short staircase. Essentially, three determinations were made and repeated several times in various different sequences (see Fig. 9): (a) of the effect at the low steady heart rate of brief (5 sec) enhancement or reduction of external calcium, e.g. by the concentration step $\Delta[Ca]_0$ of ± 1 (or 1·5) mM, where the increment or decrement of tension, $\pm \delta\psi_1$, with respect to the steady twitch tension, ψ_1, served as a measure of the effect; (b) of the size, ψ_2, to which twitches rose during the short staircase, i.e. that of the third twitch after enhancement of the heart rate from 4 to 12 min^{-1}; and (c) of the increment or decrement, $\pm \delta\psi_2$, by which the same concentration step, $\pm \Delta[Ca]_0$, as in a altered the size of the third twitch of a staircase (induced as in b). On the first hypothesis the expected result is

that the two increments or decrements $|\delta\psi_1|$ and $|\delta\psi_2|$ should be approximately equal, since the effect of the concentration step, $\pm\Delta[Ca]_0$, should not greatly depend on the residual level of calcium outside or inside the cell, whereas it would be expected on the second hypothesis that $|\delta\psi_2| > |\delta\psi_1|$. This is because a mechanism which facilitates the calcium
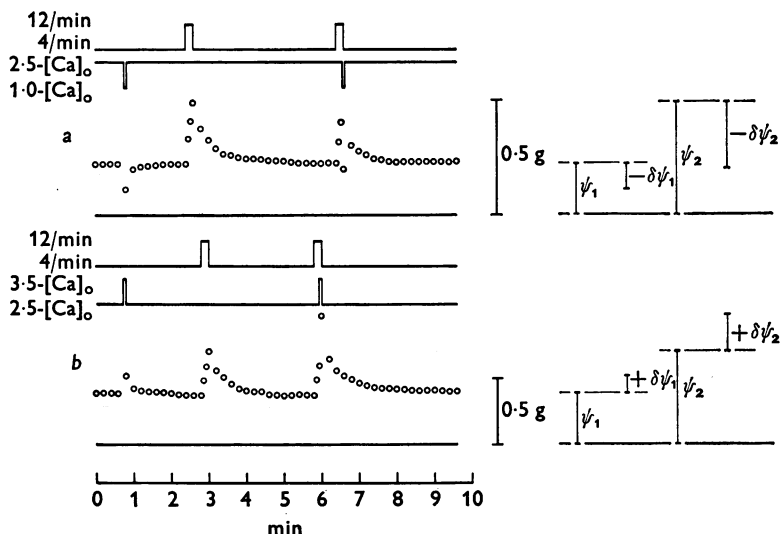

Fig. 9. Effects of transient changes of $[Ca]_0$ on twitch heights recorded either at a low regular heart rate or at the height of a short staircase. *a*. Tracings of twitch records (levels of P_{max}) illustrating the determinations (i) of the twitch height, ψ_1, at a steady heart rate (5 min^{-1}) ($[Ca]_0 = 2\cdot5$ mM); (ii) of the tension decrement, $-\delta\psi_1$, due to reduction of $[Ca]_0$ by $1\cdot5$ mM (for 4 sec before the test twitch and an additional 1 sec period which included the duration of the test twitch itself); (iii) of the height, ψ_2 of the third twitch of a short staircase (three beats at the rate of 12 min^{-1}); (iv) of the decrement, $-\delta\psi_2$ (with respect to ψ_2) due to reduction of $[Ca]_0$ by $1\cdot5$ mM (timing of the change of $[Ca]_0$ as for (ii)). *b*. As in *a*, but showing the tension increments, $+\delta\psi_1$, and $+\delta\psi_2$, evoked by brief increases of 1 mM in $[Ca]_0$. Different ventricle from *a*.

movements at a constant level of $[Ca]_0$, should also magnify the change of these movements in response to variation of $[Ca]_0$. Fig. 10 shows the results of a complete experiment of this kind in a plot combining the average twitch height of the low steady twitch, ψ_1, and of the third twitch of the staircase, ψ_2, with the values of the two pairs of increments or decrements, $\pm\delta\psi_1$ and $\pm\delta\psi_2$, added to and subtracted from the levels ψ_1 and ψ_2, respectively. As is seen, tension changes due to variation of $[Ca]_0$ were markedly greater in tests made at the high level of twitch tension ψ_2 than at the

lower level ψ_1, a result clearly favouring the second hypothesis of facili-
tation of calcium movements during the staircase.

It is of interest to make a quantitative comparison of the two staircase
effects examined, i.e. of the change in tension level from ψ_1 to ψ_2, and of
the increment or decrement from $|\delta\psi_1|$ to $|\delta\psi_2|$. The values obtained in the
experiment of Fig. 10 for the ratios ψ_2/ψ_1 and $\delta\psi_2/\delta\psi_1$ came to 2·0 and 1·93,
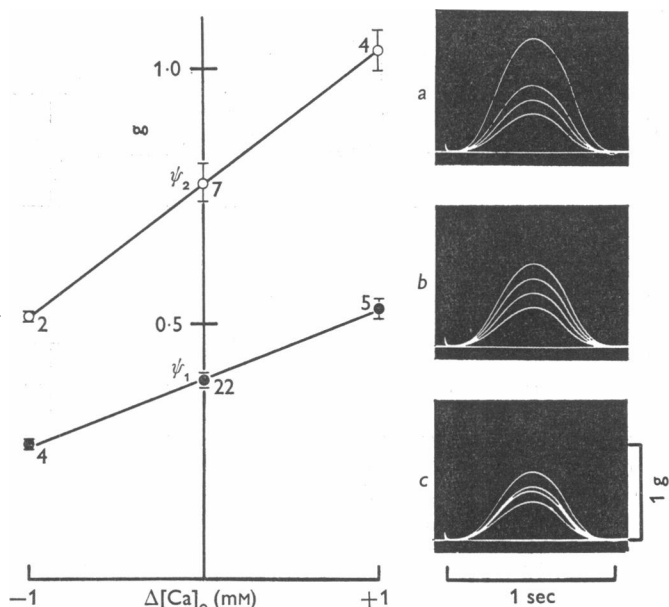

Fig. 10. Results of complete experiment of the kind shown in Fig. 9.
(Enhancement and reduction of $[Ca]_0$ from 2·5 to 3·5 and to 1·5 mM,
respectively; steady heart rate 4 min^{-1}; short staircase: three twitches at
the rate of 12 min^{-1}). L.H.S.: plot of the values ψ_1 and ψ_2, and of $(\psi_1 \pm \delta\psi_1)$
and $(\psi_2 \pm \delta\psi_2)$ against $\pm \Delta[Ca]_0$. Numbers indicate number of determina-
tions of which mean values $\pm$ S.E. are plotted, ● and ○: values determined
at steady rate, and at end of short staircase respectively. The super-
imposed twitch records (R.H.S.) are (a) of a twitch of height ψ_1 (at the steady
heart rate) followed by a short staircase of which the third twitch is
enhanced, due to $+\Delta[Ca]_0$, to the level $(\psi_2 + \delta\psi_2)$; (b) of a twitch of height
ψ_1 followed by a short staircase to the level ψ_2; (c) of a twitch of height ψ_1
followed by a short staircase of which the third twitch is reduced, due to
$-\Delta[Ca]_0$, to the level $(\psi_2 - \delta\psi_2)$ (second twitch from below). Note peak
time of twitches remain practically unaltered throughout.

respectively. This close similarity of the magnitudes of the two effects was
observed in all experiments of this kind (a total of seven) in which the
mean value of the quotient $\delta\psi_2/\delta\psi_1/\psi_2/\psi_1$ was 1·06 (S.E. $\pm 0·035$). The
result, that the height of twitches and their responsiveness to variation of
$[Ca]_0$ increased in a parallel fashion, also serves to support the second type

of hypothesis made to explain the changes in $[Ca_1]$ under present conditions. This may be discussed in relation to the following expression which was set up (Chapman & Niedergerke, 1970, eqn. (9)) to describe the dependence of twitch tension on the level of the two hypothetical compounds Ca_1 and Ca_2 (at various levels of $[Ca]_o$ but at a constant heart rate):

$$\psi = K[Ca_1]\{[Ca_2] - {}^{th}[Ca_2]\}.$$

On the assumption that variations in level of $[Ca_2]$ are negligibly small (cf. Fig. 8) and that the effect of facilitation of calcium movements is to enhance $[Ca_1]$ by a certain factor, the two variables, ψ and $\delta\psi/\delta[Ca]_o$ should
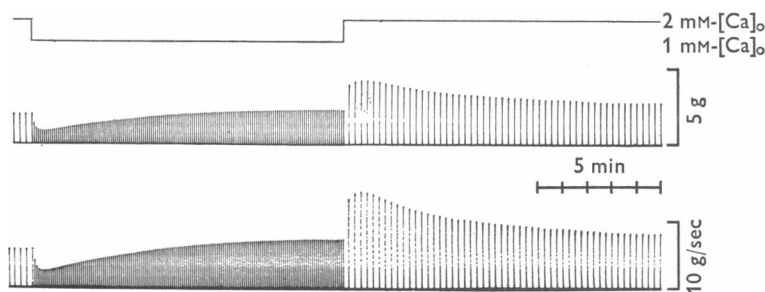

Fig. 11. Effects of simultaneous changes of $[Ca]_o$ and heart rate. Upper trace, tension record; lower trace, positive phase of differentiated tension record of twitches. The first response was to a reduction in $[Ca]_o$ from 2 to 1 mM, while at the same time the rate of stimulation was increased from 4 to 12 min^{-1}. When a new steady state had been reached, the original conditions were restored.

both increase during the short staircase by the same factor, in agreement with the above observation.

Taken together with the results of the preceding paper (Chapman & Niedergerke, 1970) the findings just described can be interpreted to suggest that there are (at least) two processes regulating the inward movements of calcium in heart cells, one of which is responsive to the calcium concentration at the cell surface, the other to the level of the intermediary I. This conclusion may, once more, be illustrated by phenomena which occur when external calcium and heart rate are varied simultaneously. In the experiment of Fig. 11 these two parameters were changed in opposite direction and in such a way as to keep (approximately) constant the steady-state twitch tension present before and after this change, e.g. by reducing $[Ca]_o$ from 2 to 1 mM while enhancing the heart rate from 4 to 12 min^{-1}, and vice versa. (In terms of Fig. 2b the transition was made, e.g. from point D to C and vice versa). As is seen in Fig. 11 a transient depression of twitch tension was obtained after reduction of calcium and facili-

tation of tension after enhancement of this concentration. This result is readily understood on the basis of the different time course in Fig. 3 of the (initial) rapid tension change after reduction of either $[Ca]_0$ or heart rate, suggesting a more rapid change of surface calcium than of the intermediary I. As a consequence of this difference, effects due to the change of external calcium should precede those due to a change in heart rate, so giving rise to the transient responses obtained. That the responses in both cases ended with slow variations in tension, a gradual rise after reduction of $[Ca]_0$ and a decline after increase of $[Ca]_0$, is explained by the fact that the initial part of the responses, i.e. up to the minimum or maximum, extended over sufficiently long periods (i.e. the time of three or four twitches) to induce variations in concentration of compound Ca_2 which are only slowly reversible.

DISCUSSION

The results of the present paper are best discussed in terms of the second of two alternative hypotheses proposed in the preceding paper (Chapman & Niedergerke, 1970) according to which the effects of two different intracellular calcium compounds, Ca_1 and Ca_2, combine to bring about the contractile process (cf. Fig. 12 and also Fig. 15, Niedergerke, 1963). Initiation of a concentration, e.g. of a twitch, is due to the formation of compound Ca_1 from calcium ions entering heart cells during the action potential; relaxation results when Ca_1 dissociates. Some of the calcium ions released from Ca_1 combine with cellular sites to form Ca_2, the remainder, we tentatively suppose, are rapidly extruded from the cell (pathway α'; although it should be noted that the evidence for such rapid calcium release is still indirect). Compound Ca_2, a 'primer' of contraction, dissociates more slowly than Ca_1 and a separate pathway may exist for the efflux of the calcium ions released (pathway γ).

On the other hand, the rapid changes which occur when the heart rate is altered are attributed to the effect of an intermediate compound I, which controls a rate-limiting step in the reaction chain leading to the formation of compound Ca_1. According to this idea, each action potential or twitch causes a transient increase of the concentration of I, so giving rise to the 'supernormal' phase of contractility, and the initial rapid portion of the ascending staircase is brought about by the gradual accumulation of I during sustained activity. Although it is clearly premature to speculate much further on the nature of this hypothetical compound, one possibility suggested by a recent result of Baker, Blaustein, Hodgkin & Steinhardt (1969) might be discussed. These authors have shown that calcium influx in the squid axon is increased by high concentrations of intracellular sodium, and it seemed possible, therefore, that accumulation

of sodium ions inside heart cells during activity might be the process facilitating calcium inward movement. This possibility was tested by following both the net loss of potassium which occurs at the beginning of continuous stimulation and the uptake of this ion after cessation of stimulation (D. C. Gadsby, R. Niedergerke & S. Page, unpublished). Potassium movements so determined are (approximate) measures of the simultaneous shifts of sodium occurring in the opposite direction, i.e. an uptake during,
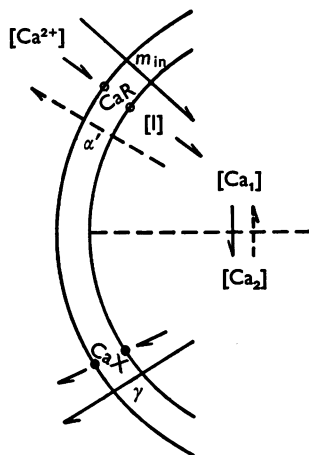

Fig. 12. Model of calcium movements in heart cells. Influx (m_{in}) of calcium ions occurs through combination with carrier molecule R and is controlled by the concentration, [I], of an intermediary. Activation of contraction is due to formation of a compound Ca_1. When Ca_1 dissociates relaxation occurs, and the calcium ions released either leave the cell via path α' or combine with other sites to form Ca_2, a 'primer' of contraction. Removal of calcium ions after dissociation from Ca_2 may involve a separate pathway (γ) (cf. also Fig. 15, Niedergerke, 1963). (The site of the intermediary I has been chosen, for the sake of definiteness, to be the cytoplasm, but no direct evidence exists to distinguish between this and the alternative possibility that the cell membrane may be concerned. In a similar way, it is tentatively assumed that formation of Ca_2 is via Ca_1, although a separate pathway may exist through which calcium ions released from the excitable membrane have direct access to the sites of compound Ca_2.)

and a loss of the ion after, activity. It was found that the time course of these potassium movements (involving about 2 m-mole K/kg tissue wet weight when a stimulus rate of 20 shocks min^{-1} is used) was quite different from that of the initial phase of the ascending and descending staircase, the half-time of K loss and uptake being between 2 and 5 min as against 5–15 sec for the rapid tension changes. It is unlikely, therefore, that the hypothetical substance I is identical to intracellular sodium.

Comparison of the present with other related results. The main feature distinguishing the present from a previous scheme (cf. discussion in con-

nexion with Fig. 15, Niedergerke, 1963) resides in the function attributed to the compound Ca_2. In both hypotheses, formation of Ca_2 and its dissociation contribute to cellular calcium movements, but Ca_2 is in the present case endowed with an important, if little understood, action in 'priming' contraction, whereas it was taken to be 'passive' in the previous scheme. However, it should also be mentioned that a passive fraction of slowly exchanging calcium (not indicated in Fig. 12) probably also exists inside the cells, possibly in equilibrium with Ca_2. This is indicated by the finding that periods of prolonged stimulation and subsequent rest are often associated with calcium movements of larger size ($> 50 \mu$-mole/l. heart cells) and with slower time course ($t_{\frac{1}{2}} > 2$–4 min) than the movements thought to be associated with the formation and decay of compound Ca_2 (Niedergerke, Page & Talbot, 1969a, b and unpublished).

Although the co-operative action of the three compounds, Ca_1, Ca_2 and the intermediary I, appears to account for the effects of changes in heart rate of the frog, well established results suggest that yet another factor must be operative in the mammalian heart. In this tissue the ascending staircase is preceded by a transient decline, and the descending staircase by a transient increase of heart beats (cf. Koch-Weser & Blinks, 1963). These additional phenomena might be related to the fact that mammalian heart cells contain considerably greater quantities of sarcoplasmic reticulum than frog heart cells, structures which, as in skeletal muscle cells, are probably stores for calcium ions released during initiation of contraction (Legato & Langer, 1969). The transient tension changes after altering the heart rate could then reflect rapid depletion or replenishment of these stores.

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